Fungal Diversity

Application of *Chaetomium* species (Ketomium®) as a new broad spectrum biological fungicide for plant disease control: A review article

K. Soytong*[1], S. Kanokmedhakul[2], V. Kukongviriyapa[3] and M. Isobe[4]

[1]Department of Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Ladkrabang, Bangkok 10520, Thailand; *e-mail: kasem_soytong@excite.com
[2]Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[3]Department of Pharmacology, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Laboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Soytong, K., Kanokmedhakul, S., Kukongviriyapa, V. and Isobe, M. (2001). Application of *Chaetomium* species (Ketomium®) as a new broad spectrum biological fungicide for plant disease control: A review article. Fungal Diversity 7: 1-15.

Research and development of biological control agents for use against plant diseases have been undertaken for several years in both government and private sectors, as natural agents are needed to take the place of chemical fungicides. The problem associated with the use of hazardous chemicals for disease control has received increasing attention worldwide, due to the fact that pathogens become resistant to chemical fungicides and the resulting environmental pollution and ecological imbalances. The implementation of practical integrated biological control technology to control plant diseases has been successfully introduced to growers in China, Philippines, Russia, Thailand and Vietnam by using new broad spectrum biological fungicides from *Chaetomium* (Thailand Patent No. 6266, International Code: AO1N 25/12 and registered as Ketomium® mycofungicide). Since 1989, the biological product has been developed and improved from 22-strains of *Chaetomium cupreum* CC01-CC10 and *Chaetomium globosum* CG01-CG12 in the form of pellet and powder formulation. The formulation has successfully been applied to infested field-soils with integrated with cultural control measures and organic amendments for the long-term protection of Durian *(Durio zibethinus* L.) and Black Pepper (*Piper nigram* L.) caused by *Phytophthora palmivora*, Tangerine (*Citrus reticulata* Blanco) caused by *Phytophthora parasitica* and Strawberry (*Fragaria* spp.) caused by *Phytophthora cactorum*, Wilt of Tomato (*Lycopersicon esculentum* L.) caused by *Fusarium oxysporum* f. sp. *lycopersici* and Basal rot of Corn (*Zea mays* L.) caused by *Sclerotium rolfsii* against Phytophthora rot. Bio-control technology of plant diseases has successfully been demonstrated, either completely or partially, when integrated with other control measures for appropriate disease management. Biological products are useful, not only for the protection against plant diseases, but can also be used for curative effects of plant diseases, and also promote plant growth.

Key words: biological control, *Chaetomium*, mycofungicide.

Introduction

Chaetomium species are normally found in soil and organic compost. *Chaetomium* is one of the largest genera of saprobic ascomycetes with more than 300 species worldwide (Arx *et al.*, 1986; Soytong and Quimio, 1989). *Chaetomium* species are potential degraders of cellulosic and other organic material and can be antagonistic against various soil microorganisms. *Chaetomium globosum* and *C. cochlioides* are antagonistic to species of *Fusarium* and *Helminthosporium* (Tveit and Moore, 1954). It has been found that by using specific strains of *C. globosum* it is possible to obtain good control over many plant pathogens. By coating seeds of corn with spores of *Chaetomium globosum* it was possible to prevent seedling blight caused by *Fusarium roseum* f. sp. *cerealis* 'graminearum' (Chang and Kommedahl, 1968; Kommedahl *et al.*, 1981). Such seed coating treatments were also found to reduce disease incidence of apple scab caused by *Venturia inequalis* (Heye and Andrews, 1983; Cullen and Andrews, 1984; Cullen *et al.*, 1984; Boudreau and Andrews, 1987). It has also been reported that some isolates of *C. globosum* produce antibiotics that can suppress damping-off of sugar beet caused by *Pythium ultimum* (Di-Pietro *et al.*, 1991). A further isolate of *C. globosum* was found to be antagonist against *Rhizoctonia solani* (Walter and Gindrat, 1988) and *Alternaria brassicicola* (Vannacci and Harman, 1987) and also reduced the quantity of sporulation of *Botrytis cinerea* on dead lily leaves exposed in the field (Kohl *et al.*, 1995). One strain of *C. cupreum* has also been reported to be antagonistic against *Phomopsis* and *Colletotrichum* spp which are soybean pathogens (Manandhar *et al.*, 1986).

Screening of *Chaetomium* species and strains isolated from soils for use as potential biological control agents commenced in Thailand in 1989. Reports indicate that strains of *C. cupreum* and *C. globosum* are able to suppress plant pathogens such as *Curvularia lunata*, *Pyricularia oryzae* and *Rhizoctonia oryzae in vitro* (Soytong, 1989, 1992a). Viable spores of *Chaetomium* spp. were able to reduce tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* in greenhouse and field trials (Soytong, 1990, 1992b) and also prevent basal stem rot of corn caused by *Sclerotium rolfsii* (Soytong, 1991). Twenty-two strains of *C. cupreum* and *C. globosum* have been found to inhibit isolates of various plant pathogens (Soytong and Soytong, 1997). These effective strains of *Chaetomium* have been formulated into bio-pellets and bio-powders for the biological control of plant diseases and have been patented and registered under the name Ketomium®. Their efficacy against tomato wilt (*F. oxysporum* f. sp. *lycopersici*) and basal rot of corn (*S. rolfsii*) has been successfully evaluated in laboratory and greenhouse tests (Soytong, 1992).

This paper reviews the development of Ketomium®, its evaluation with field trials, its patenting, biosafety testing and mechanisms of controlling disease and increasing crop production.

Field Evaluations with Ketomium®

In order to patent the *Chaetomium* strains (named Ketomium®) as a mycofungicide we carried out the following field evalutions on various crops in order to test the activity of the mixed strains prepared as biopellets and biopowders.

Tomato

Heavy reduction in tomato crop yields result from infection with *Fusarium* in Thailand (Soytong, 1992). *Fusarium*-suppression tests and pathogen suppression tests were carried out for tomato. In the *Fusarium*-suppression tests soil that was already infected with *Fusarium* was used for planting. The Ketomium® application was however applied to the soil two months before the seedlings were planted in the plot. In the pathogen suppression tests plants were grown in the *Fusarium* infected plots without the addition of Ketomium® and when disease symptoms appeared Ketomium® was applied. No Ketomium® applications were made in the control while a further trail involved the application of the chemical fungicide Pentachloronitrobenzene (PCNB). These trails are described in detail by Soytong and Soytong (1997).

Ketomium® in the form of biopellets and biopowders were applied to *Fusarium*-infested soils where tomatoes were growing and it was found that Ketomium® suppressed pathogen growth and thus disease symptoms. In *Fusarium*-suppressive soils, it was shown that the tomato plants treated with Ketomium® biopowder and the chemical fungicide PCNB completely prevented damage by *F. oxysporum* f. sp. *lycopersici*. The disease incidence in plants treated with biopellets (22%) was significantly lower than in the non-treated plants (43%). Ketomium® in biopellet and biopowder form were as effective in reducing infection rates in the tomato plants as treatment with PCNB (Soytong and Soytong, 1997).

Maize

Basal rot of maize caused by *Sclerotium rolfsii* is a serious disease in lowland fields in Thailand. Ketomium® was also effective in the control of pathogens and disease suppression when applied to maize. There was a distinct difference in disease incidence between the treated plants and the non-treated controls. In the pathogen suppression test, disease incidence in the biopellet,

biopowder and PCNB treated plants were 14.5, 15 and 16%, respectively. These were significant improvements, compared with the 23.5% disease incidence in the control. In the disease suppression tests, the same pattern was observed: disease incidence percentages of 16, 15.75 and 15.25 for the biopellet, biopowder and PCNB treated plants, respectively, compared with 25.55% in the control. It was shown that the mycofungicide, in both forms, was equally as effective as the fungicide PCNB in our experiments (Soytong and Soytong, 1997).

Tangerine

Integrated biological control of *Phytophthora* rot of Tangerine using mycofungicide under commercial growing conditions was investigated in Thailand for two years and the experiments were repeated four times. Tangerine (*Citrus reticulata*) is an important economic crop in Thailand. The major problem encountered in the citrus orchard is root and basal rots caused by *Phytophthora parasitica*. The pathogen has invaded citrus planting areas over the previous 30 years and the growers traditionally apply fungicides such as metalaxyl and alleiate. In some cases the infected citrus trees have slowly died over several years. Ketomium® has been applied to orchard soils infested with *P. parasitica and* integrated with liming to adjusting soil pH and the addition of organic compost every four months to prevent root and stem rot of Citrus. Ketomium® treatments significantly reduced disease incidence by 47.25% when compared to the fungicide (metalaxyl). Incidentally, the inoculum of *P. parasitica* in soil at a depth of 15 and 30 cm was significantly lower with Ketomium® treatment than the non-treated soils. The pathogen inoculum at the upper soil profile had a higher population than the lower soil profile. It was also observed that Ketomium® treatment produced significantly higher growth parameters in the Citrus plants than with chemical fungicide treatments. *Chaetomium* treatments also gave significantly better fruit yields (52.35 kg/plant) than the fungicide treatments (27.79 kg/plant) as shown in (Fig. 1 and Tables 1, 2).

Table 1. Disease index and disease reduction of *Phytophthora* rot of citrus after applying mycofungicide in the field.

Treatment	Disease Index[1]				Disease reduction (%)		
months	1	3	6	9	3	6	9
Chaetomium	6.71 a[2]	3.05 b	2.30 b	1.85 b	16.71	35.41	47.25
Control	6.90 a	3.40 a	3.50 a	3.90 a	-	-	-

[1]Disease index : 1 = no disease and 10 = severe disease.

[2]Average of four repeated experiments. Means with the same common letters in each column are not significantly different.

Fig. 1. Comparative study between before and after application of Ketomium® for plant disease control. (Top = citrus tree before experiment; Bottom = citrus tree after experiment).

Table 2. Percentage colonization by *Phytophthora parasitica* after applying mycofungicide in the citrus orchard

Treatment	**Colonization (%)**[1,2]											
Soil depth	15 cm						30 cm					
	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan
Chaetomium	60a	40a	40a	37a	28a	20a	46a	42a	37a	38a	27a	21a
Control	58a	55b	57b	61b	83b	93b	43a	47a	51b	55b	70b	75b

[1]Colonization (%) = number of colonized leaf discs with *P. parasitica* / total number of tested leaf discs × 100.
[2]Average of four repeated experiments. Means with the same common letters in each column are not significantly different.

Black Pepper

The application of Ketomium® in pellet form was conducted in soils infested with *P. palmivora* in Chantaburi Province, Thailand to prevent root and stem rots of Black Pepper (*Piper nigram*). The experiment was repeated four

Fig. 2. Comparative study between before and after application of Ketomium® for plant disease control. (left = black pepper before experiment, right = black pepper after experiment).

Table 3. Population dynamics of *Phytophthora* inoculum and antagonistic fungi in rhizosphere soil of Black Pepper.

Treatments	**Propagules (cfu/soil g $^{-1}$)**				**Colonization (%)** [1,2]			
Months	1	4	8	12	1	4	8	12
Chaetomium	2.7 a[2]	2 a	1.7 a	1.7 a	74.50	76.25	18	39
Metalayxl	3.1 a	2.8 b	2.2 b	1.5 a	78	87	66.50	46.75
Control	3.1 a	3.6 c	3.2 c	3.2 b	97.50	93.50	100	79.75

[1]Colonization (%) = number of colonized leaf discs with *P. palmivora* / total number of tested leaf discs × 100.
[2]Average of four repeated experiments. Means with the same common letters in each column are not significantly different.

times. Ketomium® was applied at the rate of 20 g/plant and incorporated with liming and organic compost at intervals of four months for one year. The application of Ketomium® resulted in significant disease suppression with disease incidence at 22.66%. Ketomium® treatment did not significantly reduce disease

incidence when compared to metalaxyl treatment (disease incidence 21.88%. Ketomium® and metalaxyl treated plots however, had significantly lower disease incidence than the non-treated plots which had the highest disease incidence of 71%. Inoculum of *P. palmivora* in rhizosphere soil of Black Pepper was also significantly lower following Ketomium® and metalaxyl treatments than in non-treated soils. Thus, Ketomium® treatment gave significantly higher plants (average of 208 cm), followed by metalaxyl treatment (177 cm) and non-treatment (71 cm) (Fig. 2 and Tables 3, 4).

Table 4. Disease incidence and plant stands of Black Pepper after applying mycofungicide comparison with Metalaxyl to control *Phytophthora* rot in the field.

Treatments	**Plant stands (cm)**				**Disease incidence (%)** [1,2]			
Month	1	4	8	12	1	4	8	12
Chaetomium	33.08a[2]	75.3b	145.06b	208.12b	1.57a	4.70a	7.82a	10.94a
Metalaxyl	33.61a	75.1b	134.79b	177.82b	5.47b	15.63b	17.97b	21.88b
Control	33.91a	60.4a	71.49a	81.88a	6.25b	14.84b	31.25c	71.63c

[1]Disease incidence (%) = number of infected plants/total number of tested plant (healthy and infected) × 100.

[2]Average of four repeated experiments. Means with the same common letters in each column are not significantly different.

Strawberry

Strawberry Crown Rot invaded the northern part of Thailand in the last few years. Biological control of Strawberry Crown Rot caused by *Phytophthora cactorum* using Ketomium® was evaluated in the infested soils planted with Strawberry at Fang District, Chaingmai Province and Maesai District, Chiangrai Province in Thailand during 1996 to 1997. Ketomium® mycofungicide in pellet form was applied at the rate of 0.04 g/plant added to soil media with 5 g of organic compost in plastic bags with seedlings of Strawberry before transplanting to the field. Chemical fungicide treatments served as a comparison. The percentage of dead plants and strawberry yield were not significantly different with the application of Ketomium® or chemical fungicides. The application of Ketomium® into the field-soil before planting however, resulted in significantly lower percentages of dead plants than Ketomium® treatments in plastic bags.

The population dynamics of antagonistic, plant pathogenic and saprobic fungi in rhizosphere soils of Strawberry were also evaluated for competitive growth in the treated and non-treated soils. It was found that the population of antagonistic fungi, *Chaetomium globosum*, and *C. cupreum* was significantly higher than that of the pathogen, *Phytophthora cactorum*, but this relationship was not shown in all experiments. This indicates that some limiting factors are

involved in biological control of plant diseases in the field, such as the ability of antagonists to grow, soil pH, organic matter content, soil aeration, moisture and chemical residues.

Durian

Durian is a tropical fruit tree originating from Indonesia and Malaysia and is now distributed in Thailand. The major problem encountered in Durian orchards is the low quality of yields due to *Phytophthora* rot. *Phytophthora palmivora* is one of the most economically important stem and root rot of Durian. Our evaluation of Ketomium® have demonstrated that Ketomium® can completely prevent stem and root rot of Durian seedlings grown in inoculated soils in greenhouse tests. The metalaxyl chemical fungicide treated seedlings had the highest disease prevalence while all the seedlings died in non-treated control. The integrated biological control tests were carried out from 1994-1996 in a Durian orchard in an area where the soil is infested with *P. palmivora* and the disease seriously affects Durian trees. Integrated biological control comprised good cultural practices, organic amendment, changes in soil acidity and removal of disease plant parts. This integrated control strategy was highly successful in controlling the disease in the field after application of Ketomium® at four months intervals and all treated disease plants recovered (Pechprome and Soytong, 1997).

Patenting and Registration of Ketomium®

Ketomium® a new broad spectrum mycofungicide (Int. cl. 5 AO 1 N 25/12, Thai Patent No. 6266, 20 years, date of issue - 22 February 1994: date of expiration - 21 February 2014) is a biological product of *Chaetomium globosum* strains Cg1, CG2, Cg3, Cg4, Cg5, Cg6, Cg7, Cg8, Cg9, Cg10, Cg11, Cg12 and *Chaetomium cupreum* strains Cc1, Cc2, Cc3, Cc4, Cc5, Cc6, Cc7, Cc8, CC9 and Cc10. Ketomium® was registered by the Department of Agriculture, Ministry of Agriculture and Co-operatives (No. 458/2539, 2 September 1996) using *C. cupreum* as a mycofungicide for the control of plant diseases, It has been also registered as a biological fertilizer for degrading organic matter and for induction of plant immunity and growth stimulant in Guangxi Province, China and distributed as a biological protection of plant diseases.

Development of Ketomium® Formulations and their Shelf-Life

Formulations of mycofungicides have gradually developed since 1992, and mycofungicides have now been formulated into both pellet and powder preparations (Soytong and Soytong, 1997). The 22 strains of *Chaetomium* spp.

are grown on potato dextrose agar for three weeks at room temperature (27-30 C). A spore suspension is then made and added to alginate mixture and dropped through a pasture pipette into a solution of 0.1 M calcium gluconate. The pellets are dried overnight before packaging. The pellets are ground to make biopowder before packaging (Fig. 3).

Compatibility of the *Chaetomium* strains when mixed together was initially tested and it was found that mixing did not effect activity, but had a synergistic effect. The biopellet and biopowder preparations were formulated and consist of a mixture of 22 effective strains of *C. cupreum* and *C. globosum* (Soytong, 1994). Viable spore populations were determined by dilution plate assays before pellet formation, 24 h after, and every two months for a period of one year. During this period pellets were stored at room temperature (27-30 C) in screw-capped jars. To test viability, pellets were dissolved in water with a mixture of 8×10^2 M KH_2PO_4 for dilution plating assays. Viable spore populations of the biopowder formulation was assessed in the same way. It was found that following storage the viability of propagules decreased in both formulations. There was a better survival rate in the biopellets (77%) compared to that of the biopowder formulation (57%) following three years of storage.

Biosafety tests

Acute toxicity tests of Ketomium® were carried out using the suspensions of *Chaetomium* spp. which consisted of equal concentrations of *C. cupreum* and *C. globosum* at the concentration of 9.9×10^7 spores/ml. A suspension was formulated in alginate sodium and talcum in sterilized water. The test animals were the Swiss-albino mice of both sexes weighing between 30-40 g. Mice were allocated into six groups for each sex. Mice were orally administered by gastric gavage with a Ketomium® suspension as a single bolus dose. Doses administered were divided into three levels for three mice groups (5, 10, and 20 ml/kg body weight, corresponding to 4.95×10^8, 9.9×10^8 and 1.98×10^9 spores/kg body weight) and another three groups fed with corresponding amounts of suspension without Ketomium®, served as control. After mice were fed with Ketomium® or the controrl formulation, the animals were examined for side effects hourly for 6 h in the first day and daily for another 7 d. Drug response profiles were recorded in a standardized worksheet of Nodine and Siegler (1964) where applicable. Observation was made for screening effects on the central nervous system and signs of intoxication. Drug response profiles were grouped as effects on the awareness, mood, CNS excitation, motor

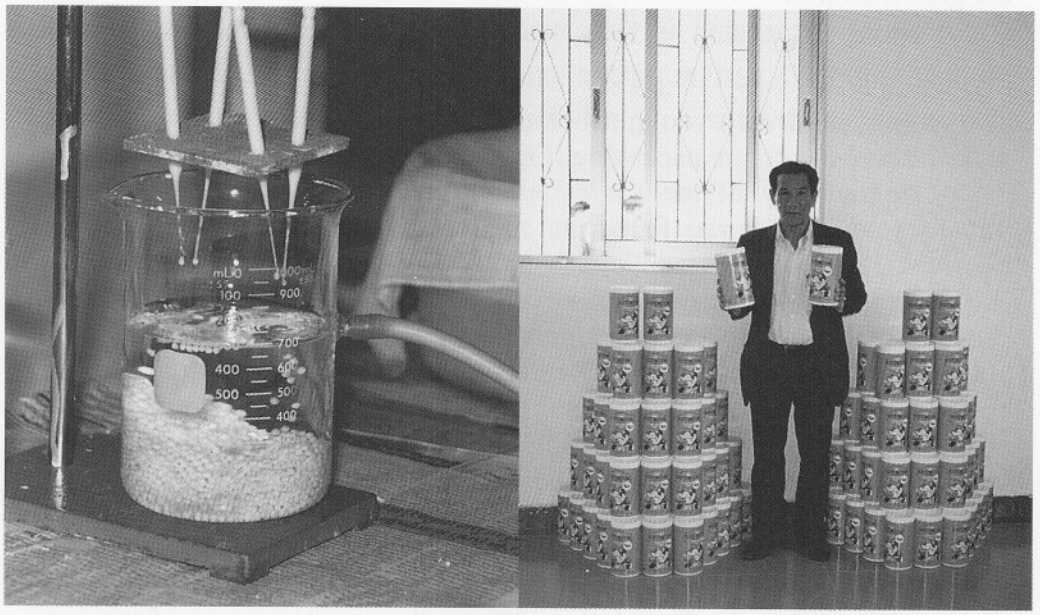

Fig. 3. Formulation of Ketomium® mycofungicide and finished product for plant disease control (left = pellet formation process, right = commercial product).

coordination, muscle tone, reflexes, autonomic signs and death. Special attention was also paid to signs of gastrointestinal disturbance, possibly due to large amount of fungal spores being fed. The number of animals that died in a 7 d period after a single dose was tabulated. In addition to mortality, weight and daily examination for signs of intoxication, lethalogy and behavioral modification were conducted. Mice were housed with four animals per cage in the animal facility of Faculty of Medicine, Khonkaen University. After the observation period, animals were sacrified and gross examination of internal organs were made and major organs were weighed.

Acute toxicity tests for Ketomium® were evaluated for bio-safety in experimental animals. It should be noted that this acute toxicity study does not ensure safety resulting from chronic exposure to spore formulations and toxicity with regard to some organ systems such as skin, lung and allergy or mutagenesis and carcinogenesis.

The values of mean lethal dose (LD^{50}) could not be obtained in this study, since there was no death resulting in experimental animals fed with the highest possible dose. It was concluded that Ketomium® shows no apparent signs of acute toxicity in experimental mice for both sexes at doses of up to 1.98×10^9 and 2×10^9 spores/ kg body weight.

How Ketomium® works

Ketomium® contains 22 strains of *C. globosum* and *C. cupreum* in the form of a spore power or pellets. *Chaetomium* species are strictly saprobic fungi belonging to the ascomycetes and are lignocellulose degraders (Arx *et al*., 1986). Specific strains of *Chaetomium* have been shown to stimulate plant growth and produce better crop yields both in the greenhouse and field, following their application as biological products (Chang and Kommedahl, 1968; Soytong and Quimio, 1989; Soytong, 1989; Soytong, 1992a; Soytong, 1992b; Pechprome and Soytong, 1997).

Control mechanisms

The mechanism of plant disease control involving specific strains of *C. globosum* and *C. cupreum* involves the production of antibiotics, e.g. *C. globosum* produces Chaetoblobosin C (Fig. 4) which suppresses the growth of plant pathogens such as *Colletotrichum gloeosporioides*, *C. dematium*, *Fusarium oxysporum*, *Phytophthora parasitica*, *P. palmivora*, *P. cactorum*,

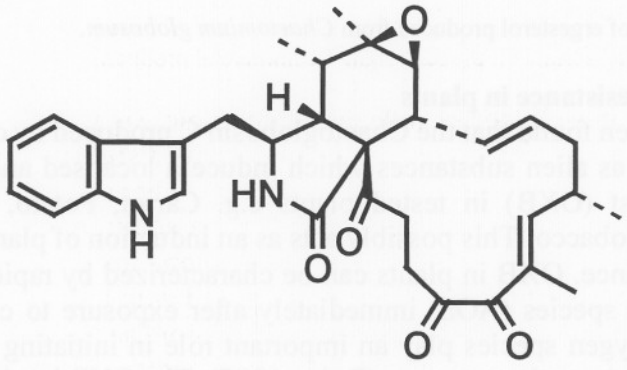

Fig. 4. Structure of Chaetoglobosin C produced from *Chaetomium globosum*.

Pyricularia oryzae, *Rhizoctonia solani* and *Sclerotium rolfsii* (Soytong and Quimio, 1989; Soytong, 1992b, Soytong and Soytong, 1997; Pechprome and Soytong, 1997).

Plant growth stimulant

It has been shown that in greenhouse and field trials, that Tomato, Corn, Rice, Pepper, Citrus, Durian, Bird's of paradise and Carnation plants treated with Ketomium®, have a greater plant growth and higher yields than non-treated plants. This implies that the mechanism of action for stimulating plant growth is probably confined to our specific strain of *Chaetomium*. It has been

found that some strains of *Chaetomium* produce substancial amounts of ergosterol (Fig. 5). This substance can help to improve the humus layer in soils which in turns leads to higher soil fertility.

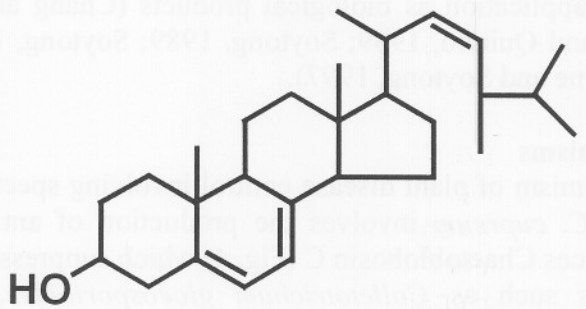

Fig. 5. Structure of ergesterol produced from *Chaetomium globosum*.

Induction of resistance in plants

It has been found that the Chaetoglobosin C produced by our strains of *C. globosum* act as alien substances which induce a localised and sub-systemic oxidative burst (OXB) in tested plants e.g. Carrot, Potato, Sweet potato, Tomato and Tobacco. This possibly acts as an induction of plant immunity for disease resistance. OXB in plants can be characterized by rapid generation of active oxygen species (AOS) immediately after exposure to certain stresses. The active oxygen species play an important role in initiating signals for the response of various plant stresses (Doke, 1997). The OXB has been postulated to be an alarm signal in plant tissues against invasion by aggressive microorganisms and it seems to act as an emergency signal for responses against attack by alien substances, and may be related to resistance or protection against plant pathogens (Doke *et al.*, 1991).

Limitations of Ketomium®, concluding remarks and future studies

The effectiveness of Ketomium® in plant disease control, especially soil-borne pathogens, integrated with other control measures such as cultural practices, e.g. sanitation, improving water drainage, pruning, removal of disease plant parts, adding organic compost and liming was investigated. These integrated control mearures completely prevented damage by various pathogens in the infested fields. The curative effects of the Ketomium® when

applied to the infected plants, combined with the manipulation of the soil environment has been surprisingly successful. Implementation of integrated biological control of plant pathogens should be used to obtain disease control and reduce economic damage and compared with the use of chemical fungicides. The potential exists to use biological techniques to modify the infested soil environment to disfavor the pathogen and increase the resistance of plants.

Our observations indicate that *Chaetomium globosum* and *C. cupreum* can adapt to harsh microclimate conditions in infested soils when applied as Ketomium® formulations. *Chaetomium* spp. have the ability to survive during dry periods and to colonize the organic substrates rapidly in the soils. Most of data on *Chaetomium* spp. have resulted from laboratory and greenhouse studies. Our studies were conducted in the field for more than four years, with the application of Ketomium® as a broad-spectrum mycofungicide. It has been shown that this biological product can be applied to the field-soils infested with the test pathogens. However, futher field trials and observations need to be carried out in order to establish a better integrated approach in using Ketomium®.

This report has shown that Ketomium® is effective and demonstrates the usefulness of an integrated approach in disease control. It discusses some limitating factors involved in biological control of plant diseases in the field such as the ability of the antagonists to grow in the field, and the effects of soil pH, content of organic matter, soil aeration, moisture and chemical residue in the soil. In different soils however, the ecology and climate may possibly result in no or poor biological control. Thus, we need to establish the biological diversity of soil microorganisms, the ecology of microbial antagonists, and the effects of chemical residues in the soil. The above experiments should also be continued in the same infested field-soils to establish whether biological control of plant pathogens can serve as long-term protection.

The expression of antagonistic activity, implying antiobiosis, is consistent with the findings of some investigators where antibiotic substances, e.g. chetomin, sterigmatocystin, chaetomin (Udagawa *et al.*, 1979; Sekita *et al.*, 1981) are released by some isolates of *Chaetomium* spp. such as *C. globosum* (Arx *et al.*, 1986). Screening for antagonism should not be limited to species, but should consider specific strains within species because these can differ in antagonistic activity (Soytong, 1992a). Several strains of *C. globosum* have been shown to produce Chaetoglobosin C which plays a role in antibiosis. These strains produce antibiotic substances which induce local and sub-systemic oxidative bursts (OXB) for signaling induction of disease resistance or immunity in plants such tomato, potato, cucumber, carrot and sweet potato.

Acknowledgements

We would like to thank N. Doke, Plant Pathology Laboratory, Department of Biological Resources and Environmental Sciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan for supporting the oxidative burst test. The research project had been fully supported from the International Foundation for Science (IFS) Sweden from 1989 to 1997 and partly supported by Third World Academy of Science (TWAS), Italy and National Research Council of Thailand. We would like to thank K.D. Hyde for his critical comments on the manuscript.

References

Arx, J.A. von, Guarro, J. and Figuera, M.J. (1986). The Ascomycete genus *Chaetomium*. Beihefte zur Nova Hedwigia 84: 1-162.

Boudreau, M.A. and Andrews, J.H. (1987). Factors influencing antagonism of *Chaetomium globosum* to *Venturia inequalis*: a case study in failed biocontrol. Phytopathology 77: 1470-1475.

Chang, I. and Kommedahl, T. (1968). Biological control of seedlings of corn by coating kernels with antagonistic microorganisms. Phytopathology 77: 1470.

Cullen, D., Berbeem, F.M. and Andrews, J.H. (1984). *Chaetomium globosum* antagonizes the apple scab pathogen, *Venturia inequalis*, under field conditions. Canadian Journal of Botany 62: 1814-1818.

Cullen, D., and Andrews, J.H. (1984). Evidence for the role of antibiosis in the antagonism of *Chaetomium globosum* to the apple scab pathogen, *Venturia inequalis*. Canadian Journal of Botany 62: 1819-1823.

Di Pietro, A., Kung, R., Gutrella, M. and Schwinn, F.J. (1991). Parameters influencing the efficacy of *Chaetomium globosum* in controlling *Pythium ultimum* damping-off of sugar-beet. Journal of Plant Disease and Protection 98: 565-573.

Doke, N. (1997). Oxidative burst: Roles in signal transduction and plant stress. In: *Oxidative Stress and the Molecular Biology of Antioxidant Defenses* Cold Spring Harbor Laboratory Press.

Doke, N., Miura, Y., Chai, H.B. and Kawakita, K. (1991). Involement of active oxygens induction of plant defense response against infection and injury. In: *Active oxygen/oxidative stress and plant metabolism* (eds E.J. Pell and K.L. Teffen). American Society of Plant Physiology, Rockville, MD: 84-96.

Heye, C.C. and Andrews, J.H. (1983). Antagonism of *Athelia bombacina* and *Chaetomium globosum* to the apple scab pathogen, *Venturia inequalis*. Phytopathology 73: 650-654.

Kohl, J.W., Molhoek, H.L., van der Plas, C.H. and Fokkema, H.J. (1995). Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field condition. Phytopathology 85: 393-400.

Kommedahl, T., Windels, C.E., Sarbini, G. and Wiley, H.B. (1981). Variability of performance of biological control and fungicidal seed treatments in corn, peas and soybean. Proceedings in Ecology 3: 5.

Manandhar, J.B., Thapliyal, N., Cavanaugh K.J. and Sinclair, J.B. (1986). Interaction between pathogenic and saprobic fungi isolated from soybean roots and seeds. Mycopathologia 98: 69-75.

Nodine, J.H., and Siegler, P.E. (1964). Animal and Clinical Pharmacologic Techniques in Drug Evaluation. Year Book Medical Publication Incorporated, Chicago, USA.

Pechprome, S. and Soytong, K. (1997). Integrated biological control of Durian stem and root rot caused by *Phytophthora palmivora*. Proceedings of the First Internatonal Symposium on Biopesticides: 228-233.

Sekita, K. Yoshihara, S., Natori, S., Udagawa, S., Muroi, T., Sugiyama, Y., Hurata, H. and Umeda, M. (1981). Mycotoxin production by *Chaetomium* spp. and related fungi. Canadian Journal of Microbiology 27: 716-722.

Soytong, K. (1989). Antagonism of *Chaetomium cupreum* to *Pyricularia oryzae*: a case study to biocontrol of a rice blast disease. *Thai Phytopathology* 9: 28-33.

Soytong, K. (1990). A taxonomic study of *Chaetomium* spp. in Thailand. Absracts, 4th Internatinal Mycological Congress, Regensburg, Germany, August 28 - September 3, 1990.

Soytong, K. (1991). Species of *Chaetomium* in Thailand and screening for their biocontrol properties against plant pathogens. Abstract.The 12th International Plant Protection Congress, Rio de Janeiro, Brazil.

Soytong, K. (1992a). Antagonism of *Chaetomium cupreum* to *Pyricularia oryzae*. Journal of Plant Protection in the Tropics 9: 17-24.

Soytong, K. (1992b). Biological control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* using *Chaetomium cupreum*. *Kasetsart Journal (Natural Science)* 26: 310-313.

Soytong, K. (1994). Development of *Chaetomium cupreum* as a soil mycofungicide for controlling tomato wilt. Abstract, 5th International Mycological Congress. August 14-21. Vancouver, British Columbia. Canada.

Soytong, K. and Quimio, T.H. (1989). Antagonism of *Chaetomium globosum* to the rice blast pathogen, *Pyricularia oryzae*. *Kasetsart Journal (Natural Science)* 23: 198-203.

Soytong, Kasem and Soytong, Kobboon. (1997). *Chaetomium* as a new broad spectrum mycofungicide. Proceedings of the 1st Internatonal Symposium on Biopesticides: 124-132.

Tveit, M. and Moore, M.B. (1954). Isolates of *Chaetomium* that protect oats from *Helminthosporium victoriae*. *Phytopathology* 44: 686-689.

Udagawa, S., Muroi, T., Kurata, H., Sekita, S., Yoshihira, K., Natori, S. and Umeda, M. (1979). The production of Chaetoglobosins, sterigmatocystin, O-methylsterigmatocystin and Chaetocin by *Chaetomium* spp. and related fungi. Canadian Journal of Microbiology 25: 170-177.

Vannacci, G. and Harman, G.E. (1987). Biocontrol of seed-borne *Alternaria raphani* and *A. brassicicola* . Canadian Journal of Microbiology 33: 850-856.

Wather, D. and Gindrat, D. (1988). Biological control of damping-off of sugarbeet and cotton with *Chaetomium globosum* or a fluorescent *Pseudomonas* sp. Canadian Journal of Microbiology 34: 631-637.

(Received 20 October 2000, accepted 10 April 2001)